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Adoptive immunotherapy of cancer
Gene transfer of T cell specificity

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Adoptive transfer of tumor-reactive T cells has emerged as a promising advance in tumor immunotherapy. Specifically, infusion of tumor-infiltrating lymphocytes has led to long-term objective clinical responses for patients with metastatic melanoma. Donor lymphocyte infusion is also an effective treatment of post-transplant lymphoproliferative disease. However, adoptive T cell therapy has restrictions in the isolation and expansion of antigen-specific lymphocytes for a large group of patients. One approach to circumvent this limitation and extend adoptive immunotherapy to other cancer types is the genetic modification of T cells with antigen-specific receptors. In this article, we review strategies to redirect T cell specificity, including T cell receptor gene transfer and antibody receptor gene transfer.

Adoptive Cell Transfer Therapy

Despite multiple approaches to therapy and prevention, cancer remains a major cause of death worldwide. Conventional therapies targeting dividing cells, using chemotherapy or radiation therapy, also affect normal cells and often fail in preventing the metastatic spread of the disease. Therefore, immunotherapy is an alternative modality of treatment that attempts to harness the specificity of the immune system to target tumor propagation without harming normal cells.[1] Active immunotherapy with therapeutic vaccines aims to elicit immune responses in vivo that will lead to an antitumor effect. With current cancer vaccine approaches, including tumor cell vaccines, peptide vaccines, viral vector vaccines, plasmid DNA vaccines and dendritic cell vaccines,[2] objective clinical responses have not yet been successfully achieved.[3] In contrast, passive immunotherapeutic strategies with adoptive cell transfer (ACT) involve ex vivo stimulation of tumor-reactive T cells that are then transferred back to the patient.[4]

Adoptive Cell Transfer Following Allogeneic Bone Marrow Transplantation

The most successful T cell immunotherapy to date has possibly been the use of donor T cells following allogeneic hematopoietic stem cell (HSC) transplantation. Indeed, treatment of patients with myeloid leukemias relapsing after allogeneic HSC transplantation by donor lymphocyte infusion has proved to induce long-term, complete remissions through graft-versus-leukemia reactivity. In this regard, the hematopoiesis-restricted minor histocompatibility antigens HA-1 or HA-2 expressed on malignant cells of the recipient can serve as target antigens for donor T cell recognition.[5,6] The role of graft-versus-leukemia effect was underscored initially by observing complete hematologic and cytogenetic remission in 3 patients with relapsed chronic myeloid leukemia infused with donor buffy coat cells.[7] Later clinical trials resulted in complete remissions in 70% of patients with chronic myeloid leukemia in response to donor lymphocyte transfusions. Complete remissions were also induced in 29% of patients with acute myeloid leukemia. In contrast, acute lymphoblastic leukemia did not respond to donor lymphocyte transfusions.[8]

During the immune recovery after HSC transplantation, reactivation of latent viruses, such as cytomegalovirus (CMV) and Epstein-Barr virus (EBV), is common and often causes an indicative disease.[9] Yet, the adoptive transfer of T cell clones appeared effective in preventing complications of virus-associated infection and disease after transplant. For example, donor, CMV-specific $CD8^+$ T cell clones were infused into recipients of the marrow transplant after stimulation with autologous fibroblasts infected with CMV. CMV-specific cytotoxic T lymphocytes (CTLs) were reconstituted and persisted in all patients.[10] Besides, CMV-specific CTLs, stimulated with CMV lysate, were successfully used in the treatment of CMV infection not responding to prolonged antiviral chemotherapy.[11] The results of adoptive immunotherapy for the prophylaxis and treatment of EBV-associated, post-transplant lymphoproliferative disease (PTLD) were also efficacious. EBV-infected B cells of PTLD are highly immunogenic, expressing latent EBV antigens including the immunodominant EBV nuclear antigen-3.[12] Moreover, lymphoblastoid cell lines generated by infecting donor B cells with a laboratory strain of EBV have effectively been used as antigen-presenting cells for the expansion of EBV-specific CTLs in vitro.[9] When donor, EBV-specific T cell lines were transferred into more than 60 marrow recipients as prophylaxis for PTLD, none of the 60 patients developed the malignancy. Of 6 additional patients that received virus-specific CTL after the onset of lymphoma, 5 patients underwent a complete regression.[13]

Overall, these studies demonstrating the efficiency of adoptive immunotherapy against allogeneic and viral antigens have

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profound implications for the development adoptive immunotherapy approaches to the treatment of patients with solid cancers.[14]

Adoptive Cell Transfer in Metastatic Melanoma

For patients with tumors that do not express viral antigens, the development of ACT therapies has had limited success, because the generation of tumor antigen-specific T cells has been problematic.[15] The finding that normal lymphocytes, after culture with IL-2, caused a significant lysis of autologous tumor led to the use of these lymphokine activated killer (LAK) cells for the treatment of patients with metastatic cancers.[16] Nevertheless, adoptive transfer of LAK cells plus IL-2 administration induced complete tumor regression in a limited number of patients.[17,18] By applying advances in basic T cell biology and new techniques, better responses have been achieved later. The adoptive transfer of tumor-infiltrating lymphocytes (TILs) into patients with melanoma along with high-dose IL-2 resulted in an objective response rate of 34%. Most of these responses, however, were transient and patients had a limited persistence of the transferred cells.[19] Another approach was to use $CD8^+$ T cell clones specific for the melanoma antigens gp100 and MART-1 for the ACT therapy, in addition to low-dose or high-dose IL-2.[20,21] Yet, T cell clones declined rapidly after adoptive transfer, and no objective clinical responses were reported. The clinical effectiveness of TIL therapy was further enhanced using nonmyeloablative, lymphodepleting chemotherapy in combination with ACT. In these protocols, patients with metastatic melanoma were infused with ex vivo-expanded, tumor-reactive TILs and high-dose IL-2 after a preconditioning regimen of cyclophosphamide and fludarabine.[22] This approach resulted in a persistent clonal repopulation of T cells and objective tumor regression in 47% of patients. A following report on the same protocol showed an objective clinical response in 51% of patients.[23] More recently, up to 72% objective response rates were observed after increasing the intensity of the preparative regimens (12 Gy plus cyclophosphamide and fludarabine).[24]

Despite encouraging results described above, the generation of tumor-reactive lymphocytes in this approach of immunotherapy is often a major limitation. Additionally, it is difficult to identify these tumor-reactive lymphocytes in many patients, in particular those with non-melanoma tumors. An attempt around this limitation is the genetic modification of T cells to redirect antigen specificity. The validity of this approach has been established by the long-term persistence of adoptively transferred, genetically modified T cells in humans.[25,26] Furthermore, a uniform population of redirected antigen-specific cells can be rapidly generated by gene transfer in accessible peripheral blood T cells, in addition to TILs. The current approaches studied in this area involve: (1) naturally occurring two-chain TCR molecules and (2) single chain antibody constructs bound to intracellular T cell signaling domains.

T Cell Receptor Gene Transfer

The main determinant of tumor recognition by T cells is appropriate binding of the major histocompatibility complex (MHC)-antigen complex by the TCR. Genes that encode the α and β chains are cloned from tumor-reactive T cells restricted to a particular HLA allele and then introduced into recipient T cells to endow them with the specificity of the donor TCR.[27] The first demonstration of redirected T cell specificity by TCR gene transfer was reported by Dembic et al. in 1986. With the identification of the TCR α and β chain sequences from two HLA-A2-restricted, MART-1/Melan A-reactive T cell clones,[29] Clay et al. were the first to report the transfer of TCR genes from one of these clones via a retroviral vector into human peripheral blood lymphocytes. Transduced cells acquired stable reactivity to MART-1/Melan A peptide and human HLA-A2 melanoma lines.[30,31] Since these studies, the same approach was used to transfer the specificity for a number of tumor-associated antigens and viral antigens associated with tumor development.[32-38]

Several viral vectors have been tested for efficient transduction of human primary T lymphocytes. Adenoviruses were the first viral vectors to be used. Although adenoviral vectors can infect both dividing and nondividing cells, they are highly immunogenic, leading to strong (or preexisting) immune responses and rapid elimination of transduced cells in vivo.[39,40] Gamma retroviral vectors were the first to provide efficient transduction. They have low immunogenicity, infect only proliferating cells and integrate into the host DNA, thus allowing stable transgene expression.[41] Different classes of retroviruses that have been used for transfer of genes to human cells include lentiviral vectors. In contrast to retroviral vectors, lentiviruses can be used to infect both dividing and nondividing cells.[42,43] Though, comparison of vectors is still lacking.

Mispairing of the introduced chains with endogenous TCR chains is a main concern in this approach; this may dilute the number of correctly expressed TCRs and could result in TCR heterodimers with unknown specificity, possibly inducing autoimmunity. Among those strategies developed to counter TCR mispainring are: (1) insertion of murine constant regions with the variable region of the human TCR[44] and (2) insertion of cysteine residues into the α and β chains that favors pairing of only the transduced chains.[45]

One concern with TCR immunotherapy is the ligand-binding affinity of TCR transgenes. Therefore, selection of a TCR with high-affinity is crucial in improving the functional avidity of TCR-transduced cells. While isolating such a TCR from a patient is rare, this obstacle has recently been overcome by using methods such as phage display. This approach has produced TCRs with picomolar affinities, representing almost a million-fold improvement in affinity compared to the parent TCR.[46] High-affinity TCRs can also be obtained from HLA-A2 transgenic mice following immunization against human cancer antigens. This method usually results in high-avidity CTLs.[47]

The first clinical example of this approach was reported by Morgan et al. at the NCI. Infusion of 15 patients with metastatic melanoma with their autologous lymphocytes transuded with genes encoding a TCR reactive against MART-1 following nonmyeloablative lymphodepletion resulted in a durable engraftment at levels exceeding 10% of peripheral blood lymphocytes for at least 2 months after treatment.[48] In this study, 2 patients (13%)

underwent objective regression of metastatic melanoma lesions. In another study, the same group attempted to use more reactive TCRs by: (1) screening highly reactive MART-1 T cell clones and (2) immunizing HLA-A2 transgenic mice with human gp100 peptide. Objective cancer regressions were seen in 30% and 19% of patients with metastatic melanoma, who received the human or mouse TCR, respectively. However, patients exhibited destruction of normal melanocytes in the skin, eye and ear, which was managed by steroid treatment.[49] More recently, autologous T lymphocytes genetically engineered to express a murine TCR against human carcinoembryonic antigen (CEA) were administered to 3 patients with metastatic colorectal cancer refractory to standard treatments. All patients showed decreased levels of serum CEA (74–99%), and one patient had an objective regression of cancer metastatic to the lung and liver. Though, a severe transient inflammatory colitis was induced in all three patients.[50]

Chimeric Antibody Receptor Gene Transfer

Chimeric antibody receptors (CARs) use the extracellular single-chain variable fragment (scFv) of an antibody fused to intracellular signaling domains, such as the TCRζ chain (CD3ζ) or IgE high-affinity receptor (FcεRIγ).[51,52] With such CARs, the recognition specificity of lymphocytes is redirected to desired cell surface tumor antigens, in a non-HLA restricted manner and without antigen processing and presentation by the target cell. Whereas a TCR binds only short peptides derived from protein antigens, CARs can recognize nonproteinous antigens, including carbohydrates and glycolipids. In addition, high-affinity scFv domain makes the transduced T cells more sensitive to low antigen densities.[53] However, a key to this approach is that the target antigens should be carefully chosen, so as to be highly expressed only on tumor cells and not on normal cells.[54]

CARs have been developed to target antigenic molecules on various human tumors, including cancers of the breast, kidney, lung, colon, prostate, ovary, skin, pancreas and immune cells.[54-60] Even though the first CARs were reported to redirect the cytotoxic function (tumor cell killing and cytokine production) of the engineered T cells, they did not transmit proliferative signals in response to antigen. To undergo full activation, T cells require signaling through their TCRs and costimulatory molecules, mainly CD28. As such, other intracellular signaling domains have been added to the CD3ζ chain of CARs. Incorporating CD28 signaling domains resulted in enhanced proliferation, cytokine secretion, and tumor control.[55,61,62] Inclusion of different costimulatory signaling domains, such as CD137 (4-1BB), CD134 (OX40) and ICOS (inducible T cell costimulator), has been shown to further augment T cell survival and effector functions through CAR recognition.[63-65]

The first CARs evaluated clinically were designed to target CD20 on lymphoma,[66] alpha-folate receptor on ovarian cancer,[67] carboxy-anhydrase-IX on renal cell carcinoma,[54] and the L1-cell adhesion molecule (CD171) and the diasialoganglioside GD2 on neuroblastoma.[68,69] Overall, these studies were characterized by short-term persistence of gene-modified T cells and poor clinical responses. In the renal cell carcinoma trial, patients developed liver toxicity, probably due to the specific reactivity of T-cells against carboxy-anhydrase-IX-expressing bile duct epithelial cells.[54] Two adverse event reports, resulting in patient deaths, have recently been published.[70,71] The first report targeted ERBB2 (HER-2/neu) in a patient with colon cancer metastatic to the lungs and liver.[70] Lymphodepletion followed by infusion of T cells transduced with a CAR vector containing CD28, 4-1BB, and CD3ζ signaling domains caused immediate pulmonary toxicity. In this study, a cytokine storm has possibly been released by the administered cells in response to the low levels of ERBB2 expressed on normal lung epithelial cells. In the second report, chronic lymphocytic leukemia patients were treated with a CAR, comprising the CD28 cytoplasmic domain in addition to that of the CD3ζ-chain.[71] One patient, who received T cells following lymphodepleting chemotherapy, developed renal failure due primarily to hypotension as a consequence of sepsis-like syndrome.

Conclusion

Infusion of antigen-specific T cells, referred to as adoptive T cell therapy, has yielded therapeutic responses in patients with chronic infections and cancer. A better understanding of how human T cells function and modulate immune responses would largely increase the therapeutic potential of adoptive cell therapy. Recently, TCR or CAR gene transfer has been developed as a reliable method to generate ex vivo large numbers of T cells of a given antigen-specificity, for which TCRs or antibodies have been identified. The ability to develop modified TCR transgenes to prevent mispairing with endogenous TCR and generate CARs incorporating multiple costimulatory signaling domains could further improve the in vivo engraftment capability and broaden the scope of the T cell gene therapy. Engineering alternate immune cell subsets, such as $CD4^+$ helper T cells, $CD4^+CD25^+$ regulatory T cells and γδ T cells to produce specialized antigen-specific T cells is a current focus of research. Though, it is also important to minimize the safety concerns accompanying the in vivo use of genetically-engineered T cells, while pursuing such therapeutic approaches.

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